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SPATIAL AND TEMPORAL PATTERNS OF MITOSIS IN THE CELLS OF THE AXIAL POLYP OF THE REEF CORAL *ACROPORA CERVICORNIS*

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Abstract

The fluorescent stain DAPI was used to observe mitoses in the endoderm and the calicoblastic ectoderm of the axial polyp of the reef coral *Acropora cervicornis*. A diel periodicity in the mitotic index (defined as the percentage of cells in some stage of mitosis) of each tissue occurred with a maximum of about 2% at midnight and a minimum of 0.5% at midday. Dividing cells were located from the tip of the column (when the polyp was contracted into the calyx) to 10 mm proximal to this point suggesting that there is no narrow zone of proliferating cells. The magnitude of the mitotic indices of these tissues suggests that it may account for the observed daily growth rate of *ca.* 300 μm in the axial polyp.

Introduction

Coral growth has been the focus of numerous studies (*e.g.*, Buddemeier and Kinzie, 1976; Highsmith, 1979; Gladfelter 1982, 1983; Wellington and Glynn, 1983); most investigators have measured some parameter of skeletal growth. Rates of tissue production have been inferred from linear increases in the skeleton (Lewis, 1982), but direct measurements have not been made. In acroporid corals, for example, an increase in the length of an axial polyp as the skeleton extends could initially involve only a change in the shape of the cells, *i.e.*, elongation. Eventually, however, the production of new tissue must involve cell division and subsequent growth.

Site and frequency of cell division have been investigated in other cnidarians, *e.g.*, *Hydra* spp. (David and Campbell, 1972; Neckelmann, 1982); colonial hydroids (Hale, 1964; Campbell, 1968); anemones (Singer, 1971; Minasian 1980); and scleractinian corals (Cheney 1973). Some authors reported that cell proliferation occurs only or at least primarily in the ectoderm (Hale, 1964; Singer, 1971; Cheney, 1973) while others stated that cell division occurs in both the endoderm and the ectoderm (Campbell, 1968; David and Campbell, 1972; Minasian, 1980). Whether this discrepancy in the site of cell proliferation is due to species specific differences or to incorrect interpretation of data has not yet been resolved (Davis, 1971).

In this study, the site and diel periodicity of mitoses in the endoderm and the calicoblastic ectoderm of the axial polyp of the reef coral *Acropora cervicornis* were determined.

Materials and Methods

Coral tips were collected at 0600, 0900, 1200, 1500, 1800, 2100, 2400, and 0200 from a depth of 11 m in Buck Island Channel, St. Croix, U. S. Virgin Islands. The tips were transferred immediately to the West Indies Laboratory. Within 30 min of

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collection, the specimens were fixed in 10% formalin and stored. The corals tips were decalcified in 10% EDTA in 0.03 *M* NaOH for a day. Each tip was trimmed to a 1 cm length. Tips were dehydrated in a graded series of ethyl alcohol, cleared in toluene, and embedded in Paraplast (m.p. 57–59°C); each step required 15 min.

Longitudinal sections, 10 μm thick were cut from prepared tissue blocks with a microtome. Sections through the midsection of the polyp were saved and placed on glass slides coated with 1% gelatin. The tissue on the slides was rehydrated (2 min per step); after 4 min in distilled water, a drop of DAPI (4′-6-Diamidino-2-Phenylindole; 1 $\mu g \cdot ml^{-1}$ distilled water; Russell *et al.*, 1975) was placed on the tissue and a coverslip placed on the slide.

The slide was examined within minutes by epifluorescence microscopy as described by Neckelmann (1982). The nuclei of all cells appear fluorescent with mitotic figures staining brightly. Zooxanthellae fluoresce red. The slide was first surveyed using the 25× objective. Fields viewed with the 63× objective were sampled from the distal tip of the polyp to 2 mm below the tip. The percentage of cells in some phase of mitosis (*i.e.*, late prophase, metaphase, anaphase, and telophase) was determined for the endoderm and the calicoblastic ectoderm for each field sampled. Enough fields were counted on each tip until *ca.* 1000 endodermal cells and *ca.* 750 calicoblastic ectodermal cells were examined. Two tips were thus examined for each time of collection and an average value determined.

Results

The tissues at the tip of the axial polyp of *Acropora cervicornis* are clearly outlined by the fluorescence of their nuclei when stained with DAPI. In these longitudinal sections, the outer ectoderm had the highest density of cells; the positions of the nematocysts and spirocysts are also clearly visible. The nuclei of the cells of the outer ectoderm overlapped so frequently that an accurate determination of cells in some phase of mitosis in this tissue layer was not feasible; some mitotic figures were observed, however.

The calicoblastic ectoderm and the endoderm appear to have a high density of cells covering the distal tip of the skeleton; as the conformation of these cells changes with distance from tip, from columnar to squamous (Gladfelter, 1983) the nuclei become spaced further apart.

Most of the nuclei in all the tissues at all times of day were in interphase (*i.e.*, some stage of the cell cycle other than mitosis). The nuclei in this condition stained brightly, but diffusely when compared to nuclei where mitotic figures were present. Occasionally, dividing zooxanthellae were also seen.

Mitosis occurs in both the endoderm and the calicoblastic ectoderm. To determine the frequency of mitosis in each tissue layer, I calculated a mitotic index (M.I.) for each tissue at each time of day. The mitotic index is the percentage of total cells in some stage of mitosis. The results, expressed as an average and a range (Fig. 1) show that the diel pattern of mitotic division in the cell populations is moderately synchronous. The M.I. of the endoderm is high (>1%) from 1800 through 0600, peaking at 2% at 2400, and low (<0.7%) from 0900 through 1500. The M.I. of the calicoblastic ectoderm also shows a peak of *ca.* 2% at 2400, but the range of values is much greater, and the peak much sharper than seen in the endoderm. All values (with the exception of almost overlapping values at 0900) are higher in the endoderm than the calicoblastic ectoderm.

Frequency of division as a function of distance from tip was not quantified. Observations indicate that dividing cells occur not only right at the tip (among the

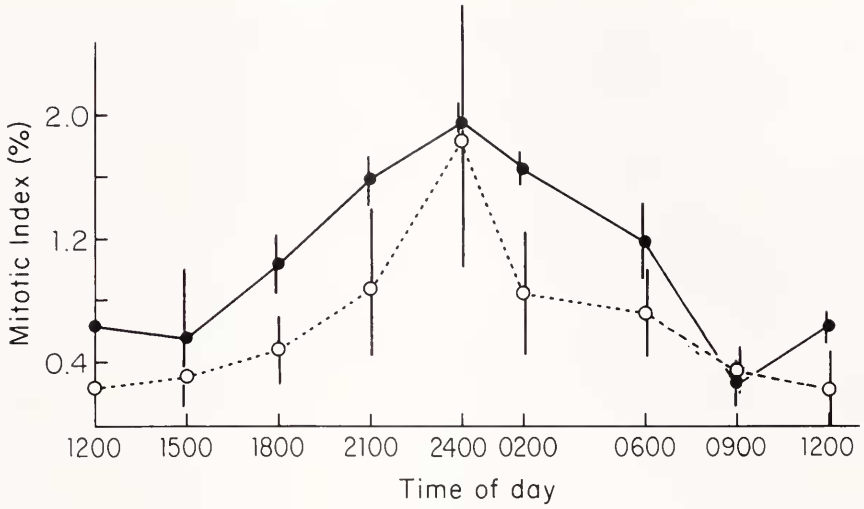

FIGURE 1. Diel pattern of percentage division (M.I.) of cells from the endoderm (solid circles) and cells from the calicoblastic ectoderm (open circles). Each point is the average of two determinations from each time period; the vertical bars indicate the range of the two values. Note that the two values for the calicoblastic ectoderm at 1500 were the same, hence no range bar.

columnar cells) but also 2 mm from the tip (the extent of the region surveyed for the determination of mitotic indices) and up to 10 mm from the tip as well. No narrow zone of cell proliferation is apparent; cells divide at random points throughout the column.

DISCUSSION

The axial polyp of *Acropora cervicornis* contains dividing cells from the tip to at least 10 mm proximal to the tip in both the endoderm and the calicoblastic ectoderm. These results are similar to those found by David and Campbell (1972) for the hydrozoan polyp *Hydra attenuata* and by Minasian (1980) for the anthozoan (actinian) polyp *Haliplanella luciae*. Both studies described proliferating cells among all epithelial layers. In addition, David and Campbell (1972) showed that the number of divisions observed in the endodermal and ectodermal tissue were enough to account for the observed growth of those cell populations; migration of cells from one epithelial layer to another probably did not occur. Until more is known about the cell cycle kinetics of *A. cervicornis*, it cannot be stated with certainty that the mitotic indices observed in this study would result in a sufficient increase in cell population to account for the observed growth rate of the polyp. However, the magnitude of the mitotic indices in the endoderm and the calicoblastic ectoderm of *A. cervicornis* is the same as that seen in *Hydra attenuata* (David and Campbell, 1972) and *H. viridis* (Neckelmann, 1982) suggesting that if the cell cycle kinetics are similar to those described for *H. attenuata* (David and Campbell, 1972), which result in a cell population doubling time of 3 days, then these observed mitotic events are probably enough to account for the rapid axial growth of *A. cervicornis*.

Campbell (1968) found that cell division occurred in both the ectoderm and the endoderm of the extending stolons of *Proboscidactyla*, a colonial hydroid. The rate of elongation along a growth axis in this situation is comparable to that seen in *A.*

cervicornis. However Hale (1964), described cell division primarily among the ectodermal cells in the stolons of another hydroid, *Clytia.* Cheney (1973) used tritiated thymidine to label proliferating cells and found labeled cells primarily among cells of the column epidermis (ectoderm) and of the polyps and coenosarc of the reef coral *Pocillopora damicornis.* The internal tissues incorporated little, if any, label. This might reflect a label uptake problem rather than a true picture of the sites of cell proliferation in coral tissues. The internal tissues probably take up label from the fluid in the coelenteron; under experimental conditions this fluid might not exchange rapidly with the external medium.

The frequency of mitosis in the endoderm and the calicoblastic ectoderm of *Acropora cervicornis* has a diel periodicity, with a peak at midnight. David and Campbell (1972) found a diel periodicity in the mitotic index of ectodermal and endodermal cells of *Hydra attenuata;* they correlated the midnight peak with a daily feeding regime at 1000 each morning. Neckelmann (1982) also found a diel periodicity in the mitotic index of endodermal cells in *H. viridis.* The peak occurred *ca.* 10–12 h after feeding; no peak occurred in starved controls. The *A. cervicornis* in the present study were collected from field populations. Normal feeding in these coral colonies probably occurs on a diel cycle set by food availability. Demersal plankton, an important food source for corals (Porter, 1974; Aldredge and King, 1977) is thought to be most abundant, *i.e.,* available for consumption, at dusk and especially at dawn (Glynn, 1973). Johannes and Tepley (1974) found that the peak feeding activity of *Porites lobata* (another reef coral with small polyps as in *A. cervicornis*) occurs at dawn. Thus the diel cycle in mitotic index of cells in the axial polyp of *A. cervicornis* might be set by a naturally occurring cycle in feeding behavior. It could also be related to the diel periodicity of a reef coral's other carbon source, *i.e.,* photosynthate transferred from the zooxanthellae (Muscatine *et al.,* 1981).

In another system in which the growth of the organism is dependent primarily on increase in cell number, *i.e.,* freshwater planarians, Baguna (1974) demonstrated a rapid increase in mitotic activity following feeding. He hypothesized that the cell population of a planarian contains a number of cells in the G2 state of the cell cycle. These cells are awaiting the proper stimulus (*e.g.,* food) to divide; the precise mechanism of how food stimulates cell division is unclear. Presumably, reef corals receiving a daily pulse of organic carbon (from zooplankton or zooxanthellae) might have a daily peak of cell division; those deprived of this normal nutritional regime should show decreased mitotic activity.

In this study, a diel cycle in mitosis was revealed. It suggests a periodicity in polyp elongation; daily extension is 300 μm. Skeletal growth in *A. cervicornis* has a diel pattern (Gladfelter, 1983). Extension in another branching acroporid is at least as rapid during the night as in the day (Barnes and Crossland, 1980). The factors which set these diel cycles in both tissue and skeletal growth are unknown.

To summarize, endodermal and calicoblastic ectodermal cells are in stages of mitosis in the column of the axial polyp of *Acropora cervicornis.* The magnitude of the mitotic indices of these cell populations are on the order of 0.5%–2% and vary in a diel pattern. Cell division in each tissue layer is probably enough for the observed rate of growth of these cell populations, resulting in a daily elongation rate of 300 μm.

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